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GC–MS/MS ANALYSES OF BIOLOGICAL SAMPLES IN SUPPORT OF EVALUATION OF TOXICITY ASSOCIATED WITH INTRAVENOUS EXPOSURE TO VX STEREOISOMERS IN GUINEA PIGS

Jeffrey M. McGuire
Linnzi K. Wright
Robert L. Kristovich

RESEARCH AND TECHNOLOGY DIRECTORATE

Michael W. Busch

EXCET, INC.
Springfield, VA 22151-2110

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This report documents the results of the gas chromatography–tandem mass spectrometry analyses of blood, tissues, and organs (heart, lung, liver, kidney, and brain) that were obtained from guinea pigs intravenously exposed to various concentrations of *O*-ethyl *S*-(2-diisopropylaminoethyl) methylphosphonothioate (VX) stereoisomers. The amount of *O*-ethyl methylphosphonofluoridate (VX-G) present in these biological samples was quantified using chemical ionization mass spectrometry with isotope dilution.

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PREFACE

The work described in this report was authorized under project no. CB10168 and Institutional Animal Care and Use Committee protocol 16-472. The work was started in January 2016 and completed in August 2016, as recorded in U.S. Army Edgewood Chemical Biological Center (ECBC; Aberdeen Proving Ground, MD) Notebook 14-0084.

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This report has been approved for public release.

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GC–MS/MS ANALYSES OF BIOLOGICAL SAMPLES IN SUPPORT OF EVALUATION OF TOXICITY ASSOCIATED WITH INTRAVENOUS EXPOSURE TO VX STEREOISOMERS IN GUINEA PIGS

1. INTRODUCTION

Many organophosphorus chemical warfare nerve agents have an asymmetric phosphorous atom, such that the synthesis of these agents results in at least two stereoisomers, designated P(+) and P(–). Although in vitro studies have demonstrated that the P(–)-isomers of these agents are several orders of magnitude more potent inhibitors of acetylcholinesterase than are the P(+)-isomers (Nordgren et al., 1984; Benschop and de Jong, 1988; Ordentlich et al., 2004), only a few in vivo studies have been conducted with optically pure stereoisomers (reviewed in Benschop and de Jong, 1988, 2001). By subcutaneously exposing mice to each of the stereoisomers of sarin, soman, tabun, and *O*-ethyl *S*-(2-diisopropylaminoethyl) methylphosphonothioate (VX), it was determined that the median lethal dose (LD_{50}) values of the P(–)-isomers of these agents were approximately half that of the racemic mixture. However, mice are not the ideal animal model for studying the toxicity associated with nerve agent exposure because they have relatively high levels of carboxylesterase activity (Maxwell et al., 1987).

The *Evaluation of the Toxicity Associated with Intravenous Exposure to the Stereoisomers of Chemical Warfare Nerve Agents in Guinea Pigs,* which is Institutional Animal Care and Use Committee (IACUC) protocol number 16-472 (IACUC, 2015), provides data to determine whether the individual stereoisomers of chemical warfare agents have different toxic responses in guinea pigs and to characterize the pharmacokinetics of the individual stereoisomers and their racemic mixtures. This report details the results of gas chromatography–tandem mass spectrometry (GC–MS/MS) analyses of blood, tissues, and organs that were performed to quantify the amounts of *O*-ethyl methylphosphonofluoridate (VX-G) present in guinea pigs after intravenous exposure to P(+)-VX, P(–)-VX, and a racemic mixture of VX.

2. METHODS

2.1 Animal Exposures

2.1.1 Animals

Adult, male guinea pigs weighing 350–400 g that had been surgically implanted with jugular vein catheters were purchased from Charles River Laboratories International, Inc. (Kingston, NY). Guinea pigs were single-housed in temperature- and humidity-controlled rooms (21 ± 1 °C and 30–70%, respectively). Lights were turned on at 0600 and off at 1800. Food and water were provided ad libitum, and guinea pigs had access to enrichment items such as huts and chew toys.

2.1.2 Range-Finding and LD_{50} Studies

Optically pure stereoisomers were separated from the racemic mixture of VX by workers at the Agent Chemistry Branch (U.S. Army Edgewood Chemical Biological Center [ECBC]; Aberdeen Proving Ground, MD) using methods described by Bae and Winemiller (2016). Guinea pigs were intravenously exposed (through their catheters) to one of the stereoisomers or the racemic mixture. A range-finding study (n = 4 or 5 animals per agent) was conducted with P(+)- and P(–)-VX to generate starting doses for the LD_{50} studies, whereas the LD_{50} value reported by Shih and McDonough (2000) was used as the starting dose for racemic VX. Initially, 2-propanol (IPA) was used as the solvent; however, this was changed to saline midway through the LD_{50} studies (exposure dates on and after 10 March 2016). In the LD_{50} studies for which IPA was the solvent (n = 9 or 10 animals per dose; injection volume of 0.5 mL/kg), the doses ranged from 100 to 1000 µg/kg for P(+)-VX, 1.8 to 3.5 µg/kg for P(–)-VX, and 7.0 µg/kg for racemic VX. In the LD_{50} studies for which saline was the solvent (n = 4–10 animals per dose; injection volume was 0.5 mL/kg), the doses ranged from 175 to 280 µg/kg for P(+)-VX, 3.0 to 5.0 µg/kg for P(–)-VX, and 3.7 to 7.0 µg/kg for racemic VX. Toxic signs were recorded continuously for 2 h post-exposure and then intermittently until the close of business. At 24 h post-exposure, guinea pigs were euthanized with a barbiturate solution containing 100 mg/kg of sodium pentobarbital. Biosamples (blood, brain, heart, liver, lung, kidneys, and urine) were collected at time of death or euthanasia.

2.1.3 Pharmacokinetic Studies

A subset of guinea pigs (n = 4 animals per dose) that were surgically implanted with double jugular vein catheters were intravenously exposed via their left catheters to the same doses of VX that were used in the LD_{50} studies with the saline solvent. Toxic signs were recorded as for range-finding studies, and guinea pigs were euthanized at 24 h post-exposure. Blood samples were collected via the right catheters at 0, 1, 10, 20, 30, 40, 50, and 60 min and 3, 6, and 24 h, and biosamples were collected at time of death or euthanasia.

2.2 Sample Preparation and Analysis

2.2.1 Chemical Materials

EA 1207 (VX-G) and deuterated ($^2H_5$) EA 1207 were obtained from ECBC stock. Before use, the EA 1207 was verified by quantitative ^{31}P NMR spectrometry to be 78.43 ± 0.56 wt % (ECBC laboratory notebook [NB] 11-0003-114), and the $^2H_5$ EA 1207 was verified to be 68.81 ± 0.9 wt % (NB 11-0003-115). Potassium fluoride (KF), IPA, ethyl acetate, glacial acetic acid, and anhydrous sodium sulfate were obtained at ≥99% purity from Sigma-Aldrich (St. Louis, MO). Sodium acetate at >99% purity was purchased from Fischer Chemicals (Fair Lawn, NJ). Ammonia and methane were obtained from Sigma-Aldrich, and helium was obtained from Messer (Malvern, PA); all were at >99.9% purity.

2.2.2 Stock Solutions and Calibration Standards

Stock solutions of VX-G and $^{2}H_5$ VX-G (internal standard [IS]) were prepared in IPA at concentrations of 1.524 mg/mL (NB 11-0003-110-01) and 1.473 mg/mL (NB 11-0003-111-01), respectively, and stored at –20 °C until use. Working solutions (5–10 µg/mL) were prepared by diluting the stock solutions in ethyl acetate. Calibration standards of VX-G were prepared by diluting the working solutions to obtain the following 12 concentration points: 0.5, 1, 5, 10, 25, 50, 100, 200, 400, 600, 800, and 1000 ng/mL (NBs 11-0003-117-04 through 11-0003-117-15). Each calibration standard also contained 200 ng/mL of $^{2}H_5$ VX-G diluted from the working solution. All calibration standards were stored at –20 °C until analysis.

For the VX-G assays, calibration curves were constructed using the 12 calibration standards from the respective analytes, where relative response (defined as $area_{analyte}/area_{IS}$) was plotted against relative concentration (defined in nanograms per milliliter as $concentration_{analyte}/concentration_{IS}$). For the VX-G calibration curve, a quadratic curve fit was used with a $1/x$ weighting factor. Typically, these calibration curves yield correlations of $R^2 = 0.999$ over 3 orders of magnitude, where R^2 is defined as the coefficient of determination.

2.2.3 Analytical Method

VX-G sample assays were performed using an Agilent 7000A Triple Quad GC/MS instrument (Agilent Technologies; Santa Clara, CA). Gas chromatographic separations were achieved using an RTx-1701 column (30 m × 0.25 mm i.d., 0–25 µm film thickness; Restek Corporation; Bellefonte, PA). The carrier gas was helium, and the flow rate was 1 mL/min. Injections of 2.0 or 3.0 µL were made using an Agilent 7693 ALS autoinjector into a splitless injector port at a temperature of 225 °C. The initial oven temperature of 35 °C was held for 6 s, then ramped to 100 °C at 15 °C/min, and ramped again at 35 °C/min to 175 °C. After each analysis was complete, the column was back-flushed at 280 °C for 4 min at reduced inlet pressure (–6.3 mL/min). The typical retention time for VX-G and its deuterated standard is 5.5 min.

Samples were ionized by positive-ion chemical ionization (CI) with ammonia reagent gas. CI source conditions were optimized using Fluoroether E3 tuning compound (Chemical Abstracts Service [CAS] registry number 3330-16-3; Agilent Technologies) with methane reagent gas. Mass spectra were obtained at a dwell time of 0.2 s for each transition in the multiple reaction monitoring (MRM) mode. Helium was used as the collision gas, and the collision energy (CE) was 12 V. The CE was optimized for the mass-to-charge ratio (*m/z*) 144 > 99 transition for VX-G and the *m/z* 149 > 100 transition for $^{2}H_5$ VX-G. The MassHunter software provided with the Agilent 7000A system was used to process and analyze the data. The software provides automated peak detection, calibration, and quantitation.

2.2.4 Sample Preparation

Sample preparations for this study were similar to those previously described by McGuire et al. (2015). Upon arrival at ECBC, all biological samples were stored at –80 °C until analysis. Whole blood samples were extracted for VX-G using Oasis HLB 30 µm solid-phase

extraction (SPE) cartridges (Waters Corporation; Milford, MA), which were first conditioned with 1 mL each of ethyl acetate, IPA, and pH 4.0 acetate buffer (0.01 M sodium acetate and 0.2 M glacial acetic acid). A sample of blood in a 2.0 mL microcentrifuge tube (Sigma-Aldrich) was weighed, and then 1 mL of acetate buffer, 200 µL of KF solution (6 M), and 1 µL of IS, $^2H_5$ VX-G were added. The mixture was vortexed for 10–20 s and centrifuged at 15,000 rpm for 5 min using a Micromax microcentrifuge (Thermo IEC; Needham Heights, MA). The supernatant liquid was transferred to the SPE cartridge, and the sediment at the bottom of the microcentrifuge tube was resuspended with 750 µL of acetate buffer and 200 µL of KF solution. This mixture was also vortex-mixed and centrifuged, and the resulting liquid was added to the original solution. After the mixture was added to the SPE cartridge, it was allowed to drain under a gentle vacuum. The analytes were eluted with 1 mL of ethyl acetate, which was collected and dried over anhydrous sodium sulfate. The ethyl acetate was withdrawn from the collection tube, filtered through a 0.2 µm nylon Acrodisc syringe filter (Pall Gelman Laboratory; Ann Arbor, MI) into a GC autosampler vial (Agilent Technologies), and then concentrated to 50 µL for analysis.

Tissue and organ sample extracts were prepared in a similar manner; freeze-fracture pulverization under cryogenic temperatures was performed before SPE extraction. A CryoPrep system (Covaris; Woburn, MA) was used to pulverize 0.5–1 g of tissue. The pulverized sample was mixed with 1 mL of acetate buffer, 200 µL of KF solution, and 1 µL of IS. This sample was then subjected to focused acoustics using an S-series focused ultrasonicator. This system directs precisely controlled cavitation and acoustic streaming to a focal point within a sample-treatment vessel in a noncontact, isothermal process. After centrifugation at 4500 rpm for 15 min using a Sorvall Legend X1R centrifuge (Thermo Fisher Scientific; Waltham, MA), the supernatant liquid was transferred to the SPE cartridge, and the sediment at the bottom of the sample tube was resuspended with 750 µL of acetate buffer and 200 µL of KF solution. This mixture was vortex-mixed and centrifuged, and the resulting liquid was added to the original solution. Additional sample processing was performed in a manner identical to that used for the blood samples.

3. RESULTS AND DISCUSSION

3.1 Range-Finding Studies

The following results were recorded in ECBC NB 14-0084. Table 1 summarizes the data from VX-G assays of whole blood, tissues, and organs that were obtained from guinea pigs after intravenous exposure to various doses of P(+)-VX. Table 2 shows similar results from intravenous exposures to various doses of P(–)-VX. The amounts of VX-G reported are representative of both free VX, which may be present, and VX bound to proteins and subsequently released as VX-G.

Although the guinea pigs were exposed to optically pure stereoisomers of VX, a racemic mixture of VX-G was obtained from all of the samples analyzed. Upon addition of KF to the acetate buffer solution, the nucleophilic substitution of F^- proceeds via an S_N1 reaction in which all stereochemistry is lost, and racemization occurs as shown in Figure 1.

Table 1. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to P(+)-VX for Range-Finding Studies

Guinea Pig No.	Exposure Date	Dose (mg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
2	27 Jan 2016	10.0*	5	5,847.3965	13,321.9823	21,393.6667	16,033.5775	22,312.9662	40,605.6615
8		1.0*	1,440	3.4910	3.2014	11.1297	106.2745	188.5999	4.4013
9		5.0*	6	3,105.2312	4,543.2631	17,736.0211	4,986.2676	11,475.2847	8,114.1826
10		2.5*	1	405.8055	1,486.9555	3,778.3479	384.9394	67.7238	445.9136
32		0.5*	2	369.4611	4,287.1277	9,959.7540	657.6626	41.9359	441.4865
215	12 Apr 2016	0.28	1,440	4.8094	16.8223	57.0460	280.9087	933.2821	13.3417
222		0.56	26	148.9941	166.1160	245.4801	1,503.0742	2,960.5377	141.3365
229		0.23	1,440	6.4527	10.8781	67.2446	92.3158	318.3802	7.6369
232		0.14	1,440	2.7106	1.4469	6.6270	32.1262	93.3448	5.0333

*2-Propanol was used as the solvent for these dosings.

Table 2. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to P(–)-VX for Range-Finding Studies

Guinea Pig No.	Exposure Date	Dose* (µg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
80	1 Mar 2016	10.0	18	10.9380	8.7204	18.5904	4.2004	18.3587	13.8842
110		1.0	1,440	0.6923	0.3571	0.5751	0.4312	BDL	1.6282
118		3.5	93	3.3024	1.8911	3.9071	10.2575	12.3515	8.7529
126		2.2	1,440	1.0952	0.4913	0.8004	0.6840	BDL	3.6986

*2-Propanol was used as the solvent for all dosings.
BDL, below detection limit (<0.05 ng/g).

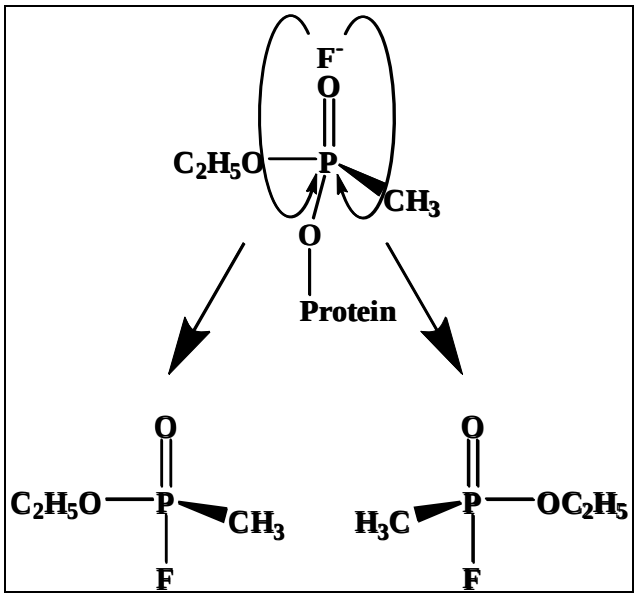

Figure 1. The S_N1 reaction: nucleophilic reagent attacks both back and front sides, resulting in racemization.

3.2 LD_{50} Studies

Table 3 summarizes the data from the VX-G assays of whole blood, tissues, and organs that were obtained from guinea pigs after intravenous exposure to various doses of P(+)-VX. Tables 4 and 5 show similar results from intravenous exposures to various doses of P(–)-VX and a racemic mixture of VX, respectively.

Figure 2 is a plot of the VX-G concentration in the blood as a function of the dose of the individual VX stereoisomers or the racemic mixture. Each point represents an average (n = 4–10 animals) of the individual values determined at the time of death or at 24 h for each dose prepared in saline. Error bars represent the standard deviation of each average determination. Curves were fitted using a Hill-type, three-parameter pharmacodynamic model of the form $y = a \times x^b/(c^b + x^b)$, where x and y represent the corresponding axis values on Figure 2, a is the maximum VX-G concentration observed, b is a sigmoidicity factor representative of the curve shape, and c is the concentration or dose that yields a 50% effect. These dose-response curves clearly show the increased toxicity of the P(–)-VX stereoisomer and the racemic mixture versus the P(+)-VX stereoisomer. From visual inspection of the curves, the dose at which a 50% response (VX-G concentration) occurred is estimated as 4–5 µg/kg for the P(–)-VX stereoisomer, 5–6 µg/kg for the racemic mixture, and 260–280 µg/kg for the P(+)-VX stereoisomer. Although these numbers cannot be construed as true LD_{50} values, they are comparable to the LD_{50} values identified by Wright et al. (2017).

Table 3. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to P(+)-VX for LD_{50} Studies

Guinea Pig No.	Exposure Date	Dose (mg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
11	27 Jan 2016	1.0*	16	367.6197	519.5762	820.5367	3,512.6174	4,128.8413	350.9682
12			15	215.1405	461.4225	674.4045	2,257.0621	5,888.4256	376.4869
13			15	447.3438	510.7501	626.7815	1,704.0373	3,107.1309	571.0728
14			13	299.8104	542.5614	746.6021	1,782.4065	2,994.5421	578.2536
16			17	292.8682	404.1579	599.5910	2,306.9841	1,754.6218	522.8988
17			13	388.9109	696.2036	862.6254	6,365.2048	1,156.4854	802.8230
19			18	269.63	357.0062	305.9527	3,382.8852	3,162.8028	668.2355
20			13	288.6617	578.7843	738.5032	1,526.3359	1,663.6786	488.7705
21			16	216.6482	616.7007	1,008.0593	775.3744	1,325.6986	643.1431
22			16	440.6334	483.3809	782.4782	2,358.8727	2,943.0750	320.3121
15		0.1*	1,440	3.8512	4.5828	17.7468	154.5606	134.2647	2.7563
23				3.5665	2.5943	10.2699	107.5471	449.6310	4.2018
24				3.7626	2.1769	9.4407	157.8269	317.7781	3.8584
25				4.0261	2.2869	12.8848	139.9483	90.0866	3.9150
26				3.0958	3.8437	23.4856	421.1333	699.7246	5.0165
27				3.4843	4.0521	37.6090	103.8152	609.2639	4.2143
28				3.1834	2.9490	17.2232	242.5674	540.1696	3.4539
29				3.6057	3.0589	13.8423	154.4708	606.1807	3.1835
30				3.5472	3.4553	11.9384	250.6466	441.2876	4.7191
31				3.3949	2.8644	11.8563	130.3585	344.4878	3.8872
33		0.5*	30	135.7689	213.0770	349.4411	1,684.4153	2,226.6943	138.0405
34			31	123.5151	154.7620	277.4501	4,308.0255	8,963.2723	111.8013
35			27	105.6681	158.0451	320.8774	2,865.1498	4,345.6519	120.4120
37			35	135.7413	137.1609	250.6580	2,477.7080	2,561.2895	93.5203
38			33	103.6892	112.8116	232.7419	2,702.8533	2,958.5497	94.8317

*2-Propanol was used as the solvent for these dosings.

(continued)

Table 3. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to P(+)-VX for LD50 Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (mg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
39	27 Jan 2016	0.5*	30	98.2962	132.6429	303.0177	1,888.2508	4,931.0791	107.3883
40			32	95.5986	130.0244	265.5998	3,413.2856	2,473.6363	88.2459
41			33	128.5214	132.5289	228.2742	2,662.1422	1,674.2905	106.7796
43			28	No sample	154.6299	259.3492	2,718.8621	8,030.0944	125.5538
44			29	117.1229	132.4259	129.8050	2,075.2998	4,815.7786	114.4125
45	28 Jan 2016	0.3*	40	90.4188	100.1918	170.6575	2,448.9813	1,911.9372	69.4550
46			48	74.1967	61.8709	114.7646	1,263.0950	1,525.4601	37.5772
48			88	37.6565	35.8046	105.0481	2,951.7492	5,814.3531	18.9562
49			46	75.4668	89.1963	184.2249	2,645.4985	1,452.8385	82.0056
50			45	71.9854	75.8710	181.1715	2,374.3413	1,485.9552	56.7199
51			42	68.5553	85.9407	179.6666	1,676.2200	2,549.1703	56.7042
52			45	67.6037	64.7845	161.8756	1,622.5549	3,533.9418	39.2166
53			39	98.1148	85.9370	184.3899	1,681.5725	960.4025	52.9353
55			40	89.2016	111.2657	227.5087	1,183.8118	2,088.2020	86.1643
57		0.15*	1,440	4.1804	10.0335	72.9183	908.6338	1,132.4542	6.0293
58				3.7631	12.2198	38.9625	944.2015	1,070.4922	5.0376
59				5.5608	13.9915	55.3750	512.5140	285.4990	5.5768
60				4.6636	12.0834	61.6722	938.4950	336.5950	6.9748
61				7.7892	10.4946	48.4688	521.0721	3,228.3681	6.1962
62				6.1386	7.5356	38.0267	366.3186	575.4307	5.7104
64				4.5203	7.6356	18.2784	146.0636	56.2294	3.8817
36				4.3186	5.7927	24.8023	441.0047	1,444.0826	4.6796
1	3 Feb 2016	0.21*	53	94.8408	75.4575	141.1850	2,096.9689	4,677.3025	72.9816
3			73	63.2023	49.5303	147.1370	1,712.1490	8,233.5432	26.5190
4			76	69.1603	43.7102	184.1990	2,652.6278	7,584.2297	27.2607
5			78	75.0011	45.8545	108.3745	2,251.4199	1,479.2198	21.9598

*2-Propanol was used as the solvent for these dosings.

(continued)

Table 3. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to P(+)-VX for LD_{50} Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (mg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
6	3 Feb 2016	0.21*	44	109.4726	80.4968	174.9116	1,206.3224	1,677.4982	60.8008
7			51	90.9247	80.3956	123.3876	1,228.2390	730.0775	53.2763
47			55	71.7622	66.5633	384.8760	2,767.4731	3,913.3086	41.8769
54			54	58.9190	57.5608	128.6920	1,780.3813	1,687.4593	41.7599
65			55	51.0443	90.5181	205.8334	2,900.4081	4,889.6125	70.7391
221	5 Apr 2016	0.175	1,440	4.8167	57.4764	154.6742	2,445.1692	1,355.4395	17.0329
223				4.0630	94.2406	366.7278	2,268.8692	2,913.9389	23.2031
224				22.3542	226.1358	796.0360	1,600.4834	2,710.0788	56.0006
225				4.7670	22..1258	91.9891	1,116.0255	979.9203	9.2807
226				4.5206	26.2746	89.8677	802.5146	448.4934	11.3057
227				4.7093	18.4959	91.3592	1,201.2346	148.1926	9.6939
228				2.8895	31.7032	87.5128	849.1210	569.1677	5.6206
230				5.7890	1.8069	6.5525	301.4764	684.5524	7.1732
231				14.7563	30.2467	231.1992	572.7788	985.0512	14.2989
233				3.4676	11.5993	56.2404	80.9442	165.1171	10.7063
234	27 Apr 2016	0.062	1,440	2.3783	1.0330	5.5495	6.1166	14.5521	1.7457
235				2.4416	0.9642	9.8437	2.6155	39.4614	1.2475
236				3.2765	1.2011	3.8910	1.8321	20.6584	1.1668
237				2.3516	1.9806	4.4907	1.7497	14.1896	1.1103
238				2.0033	1.0363	4.8924	3.0372	64.7529	1.4033
239				2.2244	1.0476	4.8973	4.5923	51.8611	1.0602
240				2.8251	0.9896	3.7095	2.0536	68.3028	1.2595
241				2.8519	1.0917	3.0039	2.6498	10.3481	0.8283
242				2.6258	0.8795	3.5483	4.3647	31.0824	0.9999
243	28 Apr 2016	0.28	35	108.5499	103.8073	450.0490	1,128.7090	5,588.0942	105.8788
244			40	77.3273	106.1143	175.8353	794.4640	3,959.6067	60.7724

*2-Propanol was used as the solvent for these dosings.

(continued)

Table 3. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to P(+)-VX for LD_{50} Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (mg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
245	28 Apr 2016	0.28	86	48.5002	107.2460	220.2025	597.8624	2,850.0274	26.4602
246			34	124.0270	101.2686	182.1559	1,470.4289	2,156.0354	94.4460
247			37	93.1963	85.5351	184.9963	4,272.7685	5,109.9987	58.3185
248			52	57.2503	51.5312	124.3433	795.9576	915.9505	50.3942
249			96	40.0441	19.0486	78.9444	1,613.5982	1,450.5470	14.4586
250			103	28.0954	17.4541	121.5443	1,014.3769	2,279.3179	18.1765
251			98	28.4870	16.7098	69.0781	1,332.9761	1,500.6994	18.0519
273	21 Jun 2016	0.21	1,440	5.9890	13.6205	30.9093	91.6437	100.7163	7.1782
274				4.5176	2.1578	12.2321	71.6553	139.9559	7.9459
275				8.2419	20.1642	109.5028	86.2091	368.9131	10.1986
276	21 Jun 2016	0.21	1,440	4.2281	15.8002	40.2624	104.3492	48.9155	8.2499
277				3.9404	4.1862	28.7462	30.3145	54.7813	4.8221
278				3.0472	35.7713	57.0586	311.5609	618.7852	11.0895
279				5.3044	24.3384	80.2497	173.3943	478.4172	10.2999
280				4.8842	7.0342	23.5295	77.8334	161.4437	7.1219
281				3.6383	6.8727	15.4113	372.6103	115.5208	6.0203
283		0.245	1,440	3.7483	19.9105	43.7904	442.0844	399.0313	11.4181
284				4.5892	11.0285	43.0022	57.3478	233.1606	8.2986
285				4.6196	15.5057	39.5968	148.7849	511.8685	9.6933
287				3.7718	19.1143	65.6033	165.4153	69.9246	9.8110
288				4.0112	10.6957	24.8123	808.7154	567.6715	8.8511
289				3.8825	8.5966	31.6284	135.9029	192.6893	6.7034
290			<1,440	No samples					
291			1,440	3.6427	14.2433	49.2973	177.2697	61.9793	9.6805
292				3.3932	26.2629	61.2158	54.7325	67.6390	6.9643
293				4.1152	15.4529	46.4755	245.6619	156.3586	8.3449

<1440, Animal died prior to 24 h.

(continued)

Table 3. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to P(+)-VX for LD_{50} Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (mg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
294	22 Jun 2016	0.26	1,440	6.2277	17.0478	47.2814	46.3610	66.0524	6.5113
297			1,440	4.7024	8.8271	9.8100	49.2839	118.2668	6.2357
298			1,440	3.5244	7.4176	15.3535	46.9616	83.2553	5.1433
299			1,440	2.3183	10.9795	19.0715	61.7500	70.5455	6.1543
300			1,440	2.8816	8.9481	20.3020	41.1902	28.6108	5.2837
301			1,440	5.8271	9.2858	18.7115	43.4706	62.0381	8.8871
302			1,398	68.7272	35.0618	18.7101	44.6110	88.3184	4.7413

Table 4. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to P(–)-VX for LD_{50} Studies

Guinea Pig No.	Exposure Date	Dose (µg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
68	1 Mar 2016	3.5[a]	30	5.3347	3.5900	6.8047	9.1295	22.9099	6.2092
79			29	5.0861	4.3729	8.8116	8.5498	27.0105	9.9515
85			37	4.0854	2.6730	4.8506	6.0027	8.9081	8.7434
92			19	5.7929	5.1043	7.7305	3.4509	16.2395	10.0177
103			29	4.8005	3.9759	9.3099	8.3823	13.2611	6.6656
108			45	5.4250	2.8958	5.6320	8.3199	9.3960	8.9735
114			23	4.9100	3.6724	8.3468	6.3332	10.2269	9.0837
120			24	4.8573	4.3357	7.6110	4.8612	20.8456	3.9985
125			37	4.7372	3.3865	5.9785	9.4440	14.1312	9.6679
66	7 Mar 2016	2.2[a,b]	1,440	0.7480	0.3604	0.6018	0.3432	0.9463	2.6316
67				1.0077	0.6987	1.3422	0.4304	1.1254	1.5899

[a]2-Propanol was used as the solvent for these dosings.
[b]Following animal exposures, NMR analyses of neat agent used to prepare these dosings indicated only 81–83% purity.

Table 4. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to P(–)-VX for LD_{50} Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (µg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
69	7 Mar 2016	2.2[a,b]	1,440	0.5695	0.3114	0.4977	0.2877	BDL	2.5807
70				0.6527	0.3346	0.4340	0.2911	0.5929	1.0903
71				0.6752	0.4133	0.5562	0.3694	BDL	2.5455
72				0.7433	0.3135	0.3577	0.2930	BDL	2.5874
74				0.7097	0.3419	0.6918	0.2664	BDL	2.0434
76				0.6999	0.2941	0.4117	0.1426	1.4507	1.8837
81				0.8296	0.3765	0.6580	0.3941	BDL	3.3260
82				0.7366	0.3782	0.7192	0.2081	BDL	2.9569
84		3.0[a,b]	1,440	0.9351	0.3934	1.0883	0.4157	0.7245	3.5956
86				0.7629	0.4074	0.7293	0.5247	BDL	3.0410
90			67	3.5862	1.4655	2.8489	2.7338	14.4339	5.992
91			1,440	0.7135	0.5821	0.8580	1.4001	1.0534	6.1766
94				1.0824	0.8750	1.2565	0.8584	1.2254	3.6520
131		3.0[a,b]	1,440	0.8807	0.5714	0.6971	0.4373	2.3775	3.3456
134				0.9230	0.3735	0.6085	0.4757	BDL	3.1044
140				0.7777	0.4407	0.8644	0.4868	BDL	4.2573
77	22 Mar 2016	3.0	1,440	0.6272	0.3436	0.6666	0.4165	BDL	3.4023
88				1.1030	0.2793	0.3418	0.5630	BDL	3.1018
93				0.8436	0.2931	0.3102	0.5717	BDL	2.1655
100				0.8473	0.4823	0.6859	0.9196	2.3387	4.7672
101				1.0259	0.4300	0.7899	0.6471	0.9119	4.6040
102				0.9023	0.4422	0.5599	0.4348	1.3578	3.0996
128				0.9120	0.2890	0.7004	0.4942	0.6432	3.7619
129				0.8065	0.3198	0.3425	0.6894	0.9009	2.9097
130				0.7579	0.4674	0.2777	0.5255	0.8636	3.2750
95		5.0	35	4.6482	2.8559	6.0981	10.5137	28.6588	8.0786

[a]2-Propanol was used as the solvent for these dosings.
[b]Following animal exposures, NMR analyses of neat agent used to prepare these dosings indicated only 81–83% purity.
BDL, below detection limit (<0.05 ng/g).

Table 4. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to P(–)-VX for LD_{50} Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (µg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
96	22 Mar 2016	5.0	181	2.9662	1.4668	3.5134	6.4265	9.4069	8.7913
105			113	3.4499	1.5913	4.9658	7.9365	12.2631	5.4513
109			134	2.9156	1.5205	2.4912	10.5164	18.0524	6.2803
113			1,440	0.9616	0.4036	0.7960	1.6326	5.3464	6.6717
132			61	4.1542	2.1711	4.4736	3.7455	13.8886	9.7702
133			172	3.3345	1.8309	4.2097	10.4734	12.8433	11.0950
137			85	2.7058	1.7109	4.2721	5.1679	35.0365	10.9229
138			36	3.7383	2.7063	4.9044	5.7604	19.0318	13.2501
97		4.0	1,440	1.0939	0.2897	0.4408	0.3764	0.4261	4.0612
115			1,440	0.6201	0.3895	0.4856	0.7341	1.0280	5.9691
116			146	2.7199	1.0828	2.4762	3.4731	10.5987	7.2884
139			1,440	4.6341	1.0809	1.4369	1.9357	27.8021	6.9294
141			146	2.6236	1.2311	4.8061	3.3888	5.7368	3.8039
147			1,440	2.9745	2.4337	4.0501	8.8499	51.7553	11.9443
148			51	0.9034	0.9463	0.7575	0.8917	7.1966	0.1800
211		4.2	1,440	0.7144	1.6111	3.2241	3.1373	61.8846	6.6070
212				0.9331	0.3575	0.4538	0.7813	16.1264	4.5767
213				0.9604	0.3067	1.8147	1.8192	23.5208	3.7092
214	22 Mar 2016	4.2	1,440	0.6444	0.4597	4.3666	0.8603	8.6556	2.5417
216			242	2.5964	1.2692	4.3196	7.7641	22.7743	7.0768
217			1,440	0.9852	0.4966	2.5123	1.8837	3.0178	4.0519
218			1,440	0.7781	0.4648	2.0600	2.6934	2.3115	5.4677
219			1,440	1.2112	1.5973	3.4611	0.5725	0.7330	1.6194
220			1,440	0.8676	0.9498	3.9424	1.2286	4.1802	5.2600

Table 5. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to Racemic VX for LD_{50} Studies

Guinea Pig No.	Exposure Date	Dose (µg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
143	7 Mar 2016	7.0*	17	15.3924	16.8396	27.8820	23.0935	116.4108	28.8881
146			15	11.0238	14.5599	40.4316	25.7339	277.5318	22.1242
149			16	10.3654	13.9037	27.3427	33.4258	43.4299	22.3774
150			13	19.6014	19.8311	58.2037	17.5263	68.7084	19.9897
152			12	12.9509	18.3767	31.7553	23.6811	251.2938	35.1998
166			19	11.2233	10.2837	22.3453	85.5740	268.0517	15.9662
167			13	16.0250	19.4756	44.4074	12.0133	243.8794	24.5218
168			18	10.3003	14.7997	37.0817	64.2143	433.8273	22.2755
78	10 Mar 2016	7.0	42	5.5961	4.5814	10.5038	31.7573	103.7902	11.0781
83			33	7.0021	5.2740	16.4919	31.9507	95.6407	10.0623
89			47	7.1829	5.3989	11.4316	58.2181	227.8316	14.1338
107			1,440	6.4610	5.5808	11.5955	43.9200	164.3720	12.0488
123			1,440	7.2369	4.6701	9.8530	35.6108	269.1729	12.6398
111		5.0	1,440	1.2676	0.5826	1.9367	7.6974	16.3417	3.5263
112				1.0183	0.5411	0.7833	3.5242	2.2788	2.8692
136				1.2161	0.7971	1.8774	5.4279	17.7490	2.3890
169			<1,440	1.4283	0.6255	1.1684	6.9638	18.6265	4.2002
170			1,440	0.9755	0.6583	3.0474	12.6399	13.8405	7.5253
171				1.1947	0.5716	1.6880	11.7517	27.3810	4.2695
172				0.4199	0.6818	3.4606	6.5627	5.5444	4.2980
75		6.0	1,440	1.2171	0.8112	2.3843	8.8715	23.3525	5.9716
119			28	3.7418	3.4580	5.8318	40.6825	143.6869	10.5019
176			1,440	1.3215	0.5127	1.8625	10.0747	19.1393	3.8866
178			1,440	1.3517	0.6316	1.6762	12.9665	22.5888	4.5305
153	23 Mar 2016	4.8	171	6.3430	0.2586	16.5318	40.3196	42.7763	9.0433

*2-Propanol was used as the solvent for this dosing. (continued)
<1440, Animal died prior to 24 h.

Table 5. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to Racemic VX for LD_{50} Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (µg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
154	23 Mar 2016	4.8	1,440	2.5391	1.1059	2.6704	8.5782	11.4143	5.0702
155		4.1	1,440	1.8622	1.4041	2.8306	7.3260	8.8541	2.3452
156				2.0305	1.4473	3.6337	4.3505	7.7146	2.3278
157				2.0672	0.7752	2.0898	6.8690	24.8946	4.6872
158				1.7007	0.9460	3.0389	5.7417	21.5219	4.2866
127		3.7	1,440	1.9959	1.3496	2.5106	4.3980	4.3915	3.7892
161				2.0462	1.6088	7.9095	15.9133	23.9412	4.3646
163				1.7888	2.0293	1.7410	4.0250	8.4988	2.5042
164				2.0050	1.1825	2.8618	8.6070	21.6200	3.7304
173				1.8237	1.0409	2.9634	8.8657	14.6460	5.2388
175				1.6983	1.4677	4.7437	5.8650	5.2279	4.8160
179				1.7956	1.3872	2.3457	6.8139	33.0112	3.2593

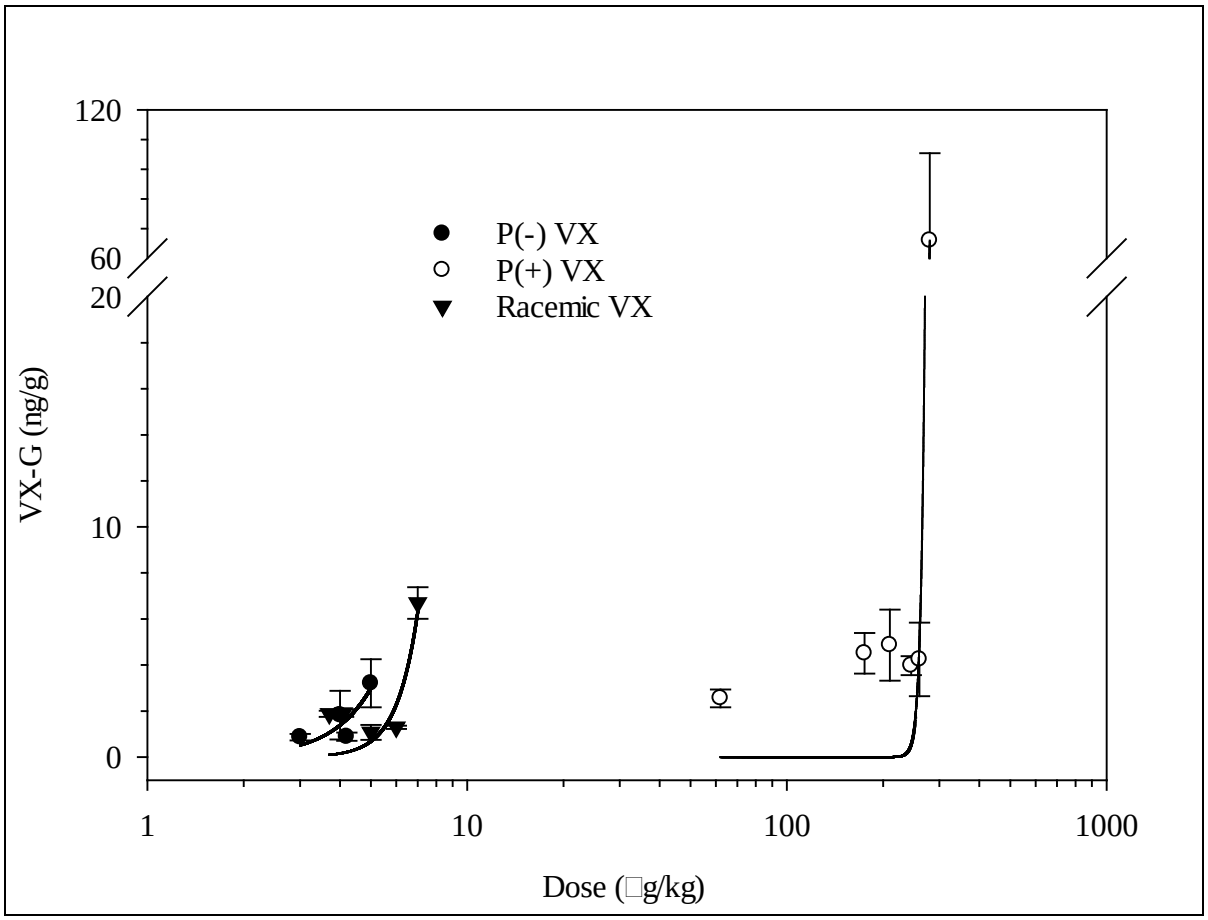

Figure 2. VX-G concentration in blood as a function of the dose of individual VX stereoisomers or the racemic mixture.

3.3 Pharmacokinetic Studies

Table 6 summarizes the data from the VX-G assays of whole blood, tissues, and organs that were obtained from guinea pigs after intravenous exposure to various doses of P(+)-VX and collection of serial blood samples. Tissues and organs were only harvested after death or at the time of euthanasia. Tables 7 and 8 show similar results from intravenous exposures to various doses of P(–)-VX and a racemic mixture of VX, respectively.

Figure 3 is a plot of the VX-G concentration in blood as a function of time for three different doses of P(+)-VX. Each point represents an average (n = 4 animals) of the individual values that were determined at the time of sampling for each dose prepared in saline. Error bars show the standard deviation of each average determination. Curves were fitted using a two-parameter pharmacokinetic model of the form $y = a \times e^{-b(x)}$, where x and y represent the corresponding axis values on Figure 3, a is the VX-G concentration at time 0, and b is the elimination rate constant (which is the same as k in Table 9). This assumes that after administration of an intravenous bolus of VX, the transfer of VX from the blood (as measured by the VX-G concentration) follows first-order kinetics. Similar data analyses were performed from serial samplings after intravenous exposures to various doses of P(–)-VX and a racemic mixture

of VX. Table 9 summarizes the elimination rate constants (k, in inverse minutes) for the various doses of VX stereoisomers and the racemic mixture as determined by the slope of the pharmacokinetic models. The half-life ($t_{½}$) of VX (as VX-G) remaining can be calculated as $t_{½} = \ln(2)/k$, as is also shown. The data revealed that a much slower clearance rate (15–20 times slower) occurred for the P(–)-VX stereoisomer and the racemic mixture, which correlates with the higher toxicity of the P(–)-VX stereoisomer and the racemic mixture.

Table 6. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to P(+)-VX for Pharmacokinetic Studies

Guinea Pig No.	Exposure Date	Dose (mg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
205	12 Apr 2016	0.175	0	No samples received					
			1						
			13	47.3661	90.4690	161.9064	1,479.0200	2,744.6300	82.4140
207			0	ISx	No samples received				
			1						
			10						
			30						
			40	Sample assays were lost during preparation					
			50						
			60						
			180						
			360						
			1,440	1.8742	8.8404	16.0046	553.1206	302.1590	7.3588
252	1 Jun 2016	0.175	0	BDL	No samples received				
			1	52.8231					
			10	32.6265					
			20	27.6206					
			30	20.3389					
			40	17.9902					
			50	15.2675					
			60	12.9064					
			180	5.7845					
			360	5.0676					
			1,440	3.0659	15.7813	36.9404	73.8697	66.5627	6.5117

BDL, below detection limit (<0.05 ng/g). (continued)
ISx, an insufficient amount of sample was received for assay.

Table 6. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to P(+)-VX for Pharmacokinetic Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (mg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
254	1 Jun 2016	0.175	0	BDL	No samples received				
			1	ISx					
			10	33.2948					
			20	33.1621					
			30	ISx					
			40	31.8474					
			50	33.0211					
			64	16.7881	23.3540	40.7594	2,726.6284	1,107.5617	23.2660
268		0.28	0	BDL	No samples received				
			1	ISx					
			10						
			20	46.3713					
			30	32.5281					
			40	ISx					
			50						
			60						
			203	20.6665	7.2703	53.0362	776.0446	826.5385	9.0034
270			0	BDL	No samples received				
			1	107.9733					
			10	52.7423					
			20	40.5516					
			30	39.0246					
			40	28.4175					
			50	25.0706					
			60	19.1688					
			180	14.5818					
			360	11.6060					

BDL, below detection limit (<0.05 ng/g).
ISx, an insufficient amount of sample was received for assay.

(continued)

Table 6. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to P(+)-VX for Pharmacokinetic Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (mg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
271	1 Jun 2016	0.28	0	BDL	No samples received				
			1	103.8394					
			10	59.9017					
			20	58.2990					
			30	ISx	No samples received				
			40						
			63		30.0360	93.7820	516.6617	212.8916	28.6461
272			0	BDL	No samples received				
			1	75.9267					
			10	34.0414					
			20	25.3866					
			30	22.6829					
			40	19.7902					
			50	14.8970					
			60	13.2719					
			180	6.1499					
			360	4.6188					
262	13 Jun 2016	0.21	1	33.4396	No samples received				
			10	27.1744					
			20	22.8026					
			50	28.1692					
			60	ISx					
			153		31.8201	57.3465	516.5460	1,425.8684	11.6506

BDL, below detection limit (<0.05 ng/g).
ISx, an insufficient amount of sample was received for assay.

(continued)

Table 6. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to P(+)-VX for Pharmacokinetic Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (mg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
266	13 Jun 2016	0.21	1	63.9592	No Samples Received				
			10	34.1746					
			20	26.5895					
			30	21.8265					
			40	19.5429					
			50	16.6621					
			60	16.4341					
			180	11.2967					
			360	Samples lost					
			1,440		2.9776	25.1167	323.5490	518.2482	6.6615
269			10	ISx	No samples received				
			30	29.3581					
269			40	ISx	No samples received				
			50	19.2091					
			60	24.4821					
			239	BDL	7.4591	39.3587	248.0948	467.3637	8.8173
306	29 Jun 2016	0.26	0	BDL	No samples received				
			10	68.4203					
			20	ISx					
			30	42.1468					
			40	ISx					
			50	31.8987					
			60	25.8275					
			180	ISx					
			335	20.6178	7.8376	37.7228	408.5275	229.3400	6.7097

BDL, below detection limit (<0.05 ng/g).
ISx, an insufficient amount of sample was received for assay.

(continued)

Table 6. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to P(+)-VX for Pharmacokinetic Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (mg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
308	29 Jun 2016	0.26	0	10.1510	No samples received				
			1	11.4679					
			10	22.2821					
			20	35.5853					
			30	32.9902					
			40	25.1858					
			50	24.6068					
			60	47.2548					
			180	70.9735					
			360	0.6111					
			1,440	4.3191	1.8057	14.6519	106.9136	144.5937	4.3381
309			0	BDL	No samples received				
			1	118.6662					
			10	50.5825					
			20	38.3982					
			30	29.6539					
			40	25.0645					
			50	22.9471					
			60	19.4047	No samples received				
			180	7.8441					
			360	7.6641					

BDL, below detection limit (<0.05 ng/g).

(continued)

Table 6. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to P(+)-VX for Pharmacokinetic Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (mg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
310	29 Jun 2017	0.26	0	BDL	No samples received				
			1	111.6205					
			10	49.2589					
			20	35.8388					
			30	30.1986					
			40	21.9792					
			50	19.8471					
			60	16.0746					
			360	7.1977					
			1,440	9.2727	36.9375	233.8527	121.2871	73.4495	6.7068
311		0.245	0	BDL	No samples received				
			1	ISx					
			10	31.5900					
			20	25.6230					
			30	23.5765					
			40	18.4448					
			50	17.3423					
			60	13.7759					
			180	5.6444					
			360	5.4667					
			1,440	3.8136	1.6437	5.3239	65.1485	37.7119	2.6862

BDL, below detection limit (<0.05 ng/g).
ISx, an insufficient amount of sample was received for assay.

(continued)

Table 6. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to P(+)-VX for Pharmacokinetic Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (mg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
303	6 Jul 2016	0.245	1	119.8871	No samples received				
			10	51.6296					
			20	43.0279					
			30	34.3514					
			40	28.5456					
			50	27.5613					
			60	ISx					
303			180	9.3291	No samples received				
			360	6.5514					
			1,440	4.2221	1.7756	12.7932	78.9552	51.0077	5.6433
304			0	BDL	No samples received				
			1	110.2506					
			10	53.0537					
			20	40.8154					
			30	35.4353					
			40	30.8270					
			50	26.1509					
			60	ISx					
			180	14.4464					
			360	7.8400					
305			1	85.0553	No samples received				
			10	45.3622					
			20	40.0229					
			30	33.2793					
			40	32.4854					
			50	34.9095					

BDL, below detection limit (<0.05 ng/g).
ISx, an insufficient amount of sample was received for assay.

(continued)

Table 6. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to P(+)-VX for Pharmacokinetic Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (mg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
305	6 Jul 2016	0.245	60	ISx	No samples received				
			180	8.5876					
			360	4.9088					
			1,440	3.7082	2.0069	8.8287	74.9796	53.2529	5.9840
307		0.21	1	99.6083	No samples received				
			10	53.9137					
			20	38.4248					
			30	37.0028					
			40	26.5192					
			50	26.2970					
			60	18.9385					
			180	8.9507					
307		0.21	1,440	7.2095	No samples received				
312		0.175	1	ISx	No samples received				
			10						
			20						
			30						
			40						
			50						
			60						
			180						
			360						
			1,440	4.7165	8.8235	18.0489	40.4379	33.0470	6.3909

ISx, an insufficient amount of sample was received for assay.

Table 7. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to P(–)-VX for Pharmacokinetic Studies

Guinea Pig No.	Exposure Date	Dose (µg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
188	5 Apr 2016	4.2	0	BDL	No samples received				
			1	ISx					
			10	2.3125					
			20	3.4517					
			30	2.6101					
			40	2.5747					
			50	2.8179					
			60	2.6433					
			180	2.2861					
			360	1.9649					
			1,440	0.7280	0.3518	0.4533	0.9912	2.8583	3.8347
189			0	BDL	No samples received				
			1	2.3679					
			10	2.2212					
			20	2.3780					
			30	2.4598					
			40	2.7345					
			50	2.6416					
			60	2.5074					
			180	2.2846					
			360	1.7257					

BDL, below detection limit (<0.05 ng/g).
ISx, an insufficient amount of sample was received for assay.

(continued)

Table 7. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to P(–)-VX for Pharmacokinetic Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (µg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
192	5 Apr 2016	4.2	0	111%*	No samples received				
			1	2.6712					
			10	3.1222					
			20	3.3248					
			30	3.1491					
			40	2.2934					
			50	2.8705					
			60	3.0374					
			180	2.5376					
			360	2.1062					
			1,440	0.8312	0.3891	0.4352	0.5124	1.3448	3.0172
194			0	ISx	No samples received				
			20	ISx					
			40	ISx					
			50	3.1004					
			60	2.9951					
			180	2.7689					
			360	2.1006					
			1,440	0.8987	0.4244	0.5582	0.9501	0.8233	6.3418

*This sample was spiked with VX-G to determine percentage recovered from assay. (continued)
ISx, an insufficient amount of sample was received for assay.

Table 7. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to P(–)-VX for Pharmacokinetic Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (µg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
209	5 Apr 2016	4.0	0	BDL	No samples received				
			1	1.1506					
			10	2.4687					
			20	2.8329					
			30	2.8066					
			40	2.9751					
			50	2.6572					
			60	2.7573					
			180	2.4735					
			360	2.0305					
			1,440	0.7594	0.3252	0.3970	0.7829	1.1482	4.2660
253	31 May 2016	3.0	0	BDL	No samples received				
			1	2.1660					
			10	2.0163					
			20	2.0864					
			30	1.9685					
			40	1.6704					
			50	1.8538					
			60	1.9363					
			180	1.5611					
			360	1.4948					
			1,440	0.3707	0.2453	0.6178	0.4852	1.5160	2.1775

BDL, below detection limit (<0.05 ng/g).

(continued)

Table 7. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to P(–)-VX for Pharmacokinetic Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (µg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
255	31 May 2016	3.0	0	BDL	No samples received				
			20	2.7002					
			30	2.2413					
			40	2.4665					
			50	1.8995					
			60	1.7895					
			180	1.8420					
			360	1.5622					
			1,440	0.5641	0.2625	0.3831	0.2120	0.8171	2.6638
256			0	BDL	No samples received				
			1	3.4256					
			10	2.6099					
			20	2.7143					
			30	2.8300					
			40	2.7376					
			50	2.4541					
			60	2.6456					
			180	2.2713					
			360	1.8868					
			1,440	0.5318	0.2634	0.4126	0.2845	8.1476	3.2098
257		4.0	0	BDL	No samples received				
			1	3.0384					
			10	3.0757					
			20	3.6126					
			30	3.2951					
			40	3.3537					
			50	3.2887					

BDL, below detection limit (<0.05 ng/g).

(continued)

Table 7. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to P(–)-VX for Pharmacokinetic Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (µg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
257	31 May 2016	4.0	60	3.4274	No samples received				
			180	2.7760					
			360	2.0940					
258			0	BDL	No samples received				
			1	2.9114					
			10	2.6274					
			20	3.0077					
			30	3.0697					
			40	3.2345					
			50	2.8910					
			60	2.8104					
			180	ISx					
			360	1.4351					
259			0	BDL	No samples received				
			1	2.9977					
			10	2.8593					
			20	2.9727					
			30	3.0966					
			40	2.8144					
			50	2.8352					
			60	2.6607					
			180	2.4734					
			360	1.9287					
			1,440	0.6568	0.3279	1.0142	0.2291	1.0931	3.4093

BDL, below detection limit (<0.05 ng/g).
ISx, an insufficient amount of sample was received for assay.

(continued)

Table 7. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to P(–)-VX for Pharmacokinetic Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (µg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
260	31 May 2016	5.0	0	BDL	No samples received				
			1	3.1315					
			10	3.1833					
			20	3.5888					
			30	3.6730					
			40	1.4309					
			50	3.7682					
			60	3.8095					
			180	2.9990					
			360	2.5796					
			1,440	0.7426	0.4592	0.6490	0.6488	0.5637	3.2290
261			0	BDL	No samples received				
			1	3.1648					
			10	3.7401					
			20	3.9175					
			30	3.5355					
			40	4.0766					
			50	3.7690					
			60	3.6321					
			180	2.6953					
			360	1.9450					

BDL, below detection limit (<0.05 ng/g).

(continued)

Table 7. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to P(–)-VX for Pharmacokinetic Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (µg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
264	31 May 2016	5.0	0	BDL	No samples received				
			1	3.5571					
			10	3.0994					
			20	3.0729					
			30	3.3483					
			40	3.7155					
			50	3.4073					
			60	3.2426					
			180	2.5005					
			360	2.2232					
265		3.0	0	BDL	No samples received				
			1	2.0273					
			10	2.1867					
			20	2.3503					
			30	2.3126					
			40	2.6012					
			50	2.3676					
			60	2.3346					
			180	2.0540					
265			360	1.7699	No samples received				
			1,440	0.5594	0.4004	0.3819	0.1220	4.6793	3.7362

BDL, below detection limit (<0.05 ng/g).

(continued)

Table 7. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to P(–)-VX for Pharmacokinetic Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (µg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
267	31 May 2016	5.0	0	BDL	No samples received				
			1	3.2516					
			10	3.3887					
			20	3.4344					
			30	3.2779					
			40	3.3340					
			50	3.3395					
			60	2.9954					
			167	1.8184	1.3027	2.5009	2.6029	24.6279	5.9398

BDL, below detection limit (<0.05 ng/g).

Table 8. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to Racemic VX for Pharmacokinetic Studies

Guinea Pig No.	Exposure Date	Dose (µg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
180	23 Mar 2016	4.8	0	BDL	No samples received				
			1	5.6508					
			10	4.9878					
			20	4.0480					
			30	4.9290					
			40	4.2287					
			50	4.1203					
			60	4.2309					
			180	3.8336					
			360	3.1521					
			1,440	1.6439	0.8790	1.6369	12.5498	26.5548	3.7624
182			0	BDL	No samples received				
			1	5.4642					
			10	5.8777					
			20	5.1333					
			30	5.7275					
			40	5.3119					
			50	4.7095					
			60	5.2397					
			180	4.6352					
			360	3.5663					
			1,440	1.6162	0.7596	2.7967	29.4239	10.4903	3.5369

BDL, below detection limit (<0.05 ng/g).

(continued)

Table 8. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to Racemic VX for Pharmacokinetic Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (µg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
183	23 Mar 2016	4.8	0	BDL	No samples received				
			1	5.8179					
			10	5.1014					
			20	5.5445					
			30	5.5172					
			40	5.3774					
			50	5.1176					
			60	5.1797					
			180	4.4672					
			360	3.7489					
			1,440	1.8634	1.1789	1.8316	11.0851	26.2179	6.3050
185			0	BDL	No samples received				
			1	5.9456					
			10	5.2385					
			20	4.8052					
			30	4.4030					
			40	4.4884					
			50	4.2926					
			60	3.9518					
			180	3.5226					
			360	2.9443					
			1,440	1.4662	0.6950	1.4151	15.0011	23.3111	4.7752

BDL, below detection limit (<0.05 ng/g).

(continued)

Table 8. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to Racemic VX for Pharmacokinetic Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (µg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
181	30 Mar 2016	7.0	0	BDL	No samples received				
			1	3.9543					
			10	3.4025					
			20	ISx					
			30						
			40						
			50						
			60						
			180						
			360	2.2645					
			1,440	1.0349	0.9668	2.1490	9.4032	171.7250	3.5190
184			0	ISx	No samples received				
			1						
			10						
			20	4.2198					
			30	ISx					
			40						
			50						
			60						
			180						
			360	2.5627					
			1,440	1.1731	1.7791	2.4435	10.9325	8.0347	4.2311

BDL, below detection limit (<0.05 ng/g).
ISx, an insufficient amount of sample was received for assay.

(continued)

Table 8. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to Racemic VX for Pharmacokinetic Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (µg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
186	30 Mar 2016	7.0	0	BDL	No samples received				
			1	3.5232					
			10	3.1968					
			20	3.5219					
			30	3.4886					
			40	3.5935					
			50	3.2308					
			60	3.0851					
			180	2.9320					
			360	2.4387					
			1,440	1.3646	1.6335	3.4071	26.7402	61.3619	5.3528
187			0	BDL	No samples received				
			1	3.3067					
			10	2.6189					
			20	2.7354					
			30	2.3520					
			40	2.4331					
			50	2.3667					
			60	2.8746					
			180	2.0920					
			360	1.6426					
			1,440	0.9480	1.1248	1.7438	13.4322	45.1526	3.9924
190		6.0	0	118%*	No samples received				
			1	3.5114					
			10	3.2312					
			21	3.1273	2.8458	9.9184	30.8527	54.4607	8.8111

*This sample was spiked with VX-G to determine percentage recovered from assay.
BDL, below detection limit (<0.05 ng/g).

(continued)

Table 8. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to Racemic VX for Pharmacokinetic Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (µg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
191	30 Mar 2016	6.0	0	BDL	No samples received				
			1	2.7796					
			10	2.4796					
			20	2.4575					
			30	2.8872					
			40	2.2457					
			50	2.2163					
			60	2.2071					
			180	2.0524					
			360	1.7368					
			1,440	0.9325	0.4247	0.8924	10.5911	30.5355	2.6535
193			0	BDL	No samples received				
			1	3.0049					
			10	2.7175					
			20	3.0337					
			30	2.8918					
			40	2.8594					
			50	2.6646					
			60	2.6270					
			180	2.4253					
			360	1.9892					
			1,440	1.1554	1.7355	4.0995	21.5055	116.3958	3.7823

BDL, below detection limit (<0.05 ng/g).

(continued)

Table 8. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to Racemic VX for Pharmacokinetic Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (µg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
195	30 Mar 2016	6.0	0	BDL	No samples received				
			1	3.4135					
			10	3.0525					
195			20	2.7628	No samples received				
			30	2.3390					
			40	2.4343					
			50	2.3165					
			60	2.6175					
			180	2.1845					
			360	1.8254					
			1,440	0.9291	1.4121	3.3995	23.8837	69.3294	4.2496
196		5.0	0	BDL	No samples received				
			1	4.5289					
			10	3.0838					
			20	3.3386					
			30	3.2996					
			40	2.8444					
			50	3.0163					
			60	2.9157					
			180	2.3040					
			360	2.4027					
			1,440	1.0515	0.7072	1.3290	9.0184	14.0947	4.0463

BDL, below detection limit (<0.05 ng/g).

(continued)

Table 8. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to Racemic VX for Pharmacokinetic Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (µg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
197	30 Mar 2016	5.0	0	BDL	No samples received				
			1	2.9508					
			10	2.7473					
			20	2.5805					
			30	2.5545					
			40	2.3774					
			50	2.5119					
			60	2.4025					
			180	2.0195					
			360	ISx					
			1,440	1.0087	0.4971	1.0177	7.6585	20.6375	2.3159
198			0	BDL	No samples received				
198			1	3.3165	No samples received				
			10	3.0786					
			20	2.8316					
			30	3.0009					
			40	2.6164					
			50	2.9105					
			60	2.8373					
			180	1.9559					
			360	1.6902					
			1,440	0.7815	0.5211	0.9505	8.2058	26.2812	3.5328

BDL, below detection limit (<0.05 ng/g).
ISx, an insufficient amount of sample was received for assay.

(continued)

Table 8. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to Racemic VX for Pharmacokinetic Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (µg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
199	30 Mar 2016	5.0	0	BDL	No samples received				
			1	2.2156					
			10	2.1580					
			20	2.2090					
			30	2.3118					
			40	2.5210					
			50	2.0540					
			60	2.2927					
			180	2.0414					
			360	1.4989					
			1,440	0.8579	0.4703	1.0219	7.5343	17.3610	2.6870
200		4.1	0	BDL	No samples received				
			1	2.4862					
			10	2.3898					
			20	2.3243					
			30	2.5103					
			40	2.3902					
			50	2.3546					
			60	2.2639					
			180	2.0389					
			360	1.6037					
			1,440	1.0195	0.4193	4.6074	0.6096	8.3628	2.6363

BDL, below detection limit (<0.05 ng/g).

(continued)

Table 8. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to Racemic VX for Pharmacokinetic Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (µg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
201	30 Mar 2016	4.1	0	BDL	No samples received				
			1	2.5105					
			10	2.3213					
			20	2.3041					
			30	2.3237					
			40	2.3818					
			50	2.2290					
			60	2.2223					
			180	2.0126					
			360	1.5143					
			1,440	0.9546	0.6681	0.8079	2.8509	2.7339	1.9049
202			0	BDL	No samples received				
			1	2.3394					
			10	2.3736					
			20	2.5656					
			30	2.9234					
			40	2.3611					
			50	2.4053					
			60	2.1873					
			180	1.8610					
			360	1.7075					
			1,440	0.9754	0.5325	0.8058	8.2207	16.8110	3.3287

BDL, below detection limit (<0.05 ng/g).

(continued)

Table 8. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to Racemic VX for Pharmacokinetic Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (µg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
203	30 Mar 2016	4.1	0	BDL	No samples received				
			1	2.2065					
			10	1.8914					
			20	2.1044					
			30	1.8926					
			40	1.9985					
			50	1.8519					
			60	1.8539					
			180	1.6124					
			360	1.4551					
			1,440	0.8176	0.4076	0.6700	6.3322	18.2030	2.7183
204		3.7	0	BDL	No samples received				
			10	2.2007					
			20	2.0657					
			30	1.9999					
			40	1.8301					
			50	1.8139					
			60	1.4865					
			180	1.6931					
			360	1.5708					
			1,440	0.7391	0.2631	0.4417	2.7774	1.1685	1.5870

BDL, below detection limit (<0.05 ng/g).

(continued)

Table 8. Results from VX-G Assays of Blood, Tissues, and Organs
Following Intravenous Exposure to Racemic VX for Pharmacokinetic Studies (Continued)

Guinea Pig No.	Exposure Date	Dose (µg/kg)	Sample Time (min)	VX-G (ng/g)					
				Whole Blood	Heart	Lung	Liver	Kidney	Brain
206	30 Mar 2016	3.7	0	BDL	No samples received				
			1	2.1550					
			10	2.4244					
			20	2.4614					
			30	2.3922					
			40	2.3494					
			50	2.4830					
			60	2.1063					
			180	2.0363					
			360	1.6765					
			1,440	1.0087	0.6088	1.3821	7.7758	18.9076	2.8424
208			0	BDL	No samples received				
			1	3.2942					
			10	2.7747					
			20	2.7421					
			30	2.7562					
			40	2.4678					
			50	2.6672					
208			60	2.4518	No samples received				
			180	2.3491					
			360	2.3268					
			1,440	1.1310	0.4591	0.8752	6.9101	16.3958	3.2021

BDL, below detection limit (<0.05 ng/g).

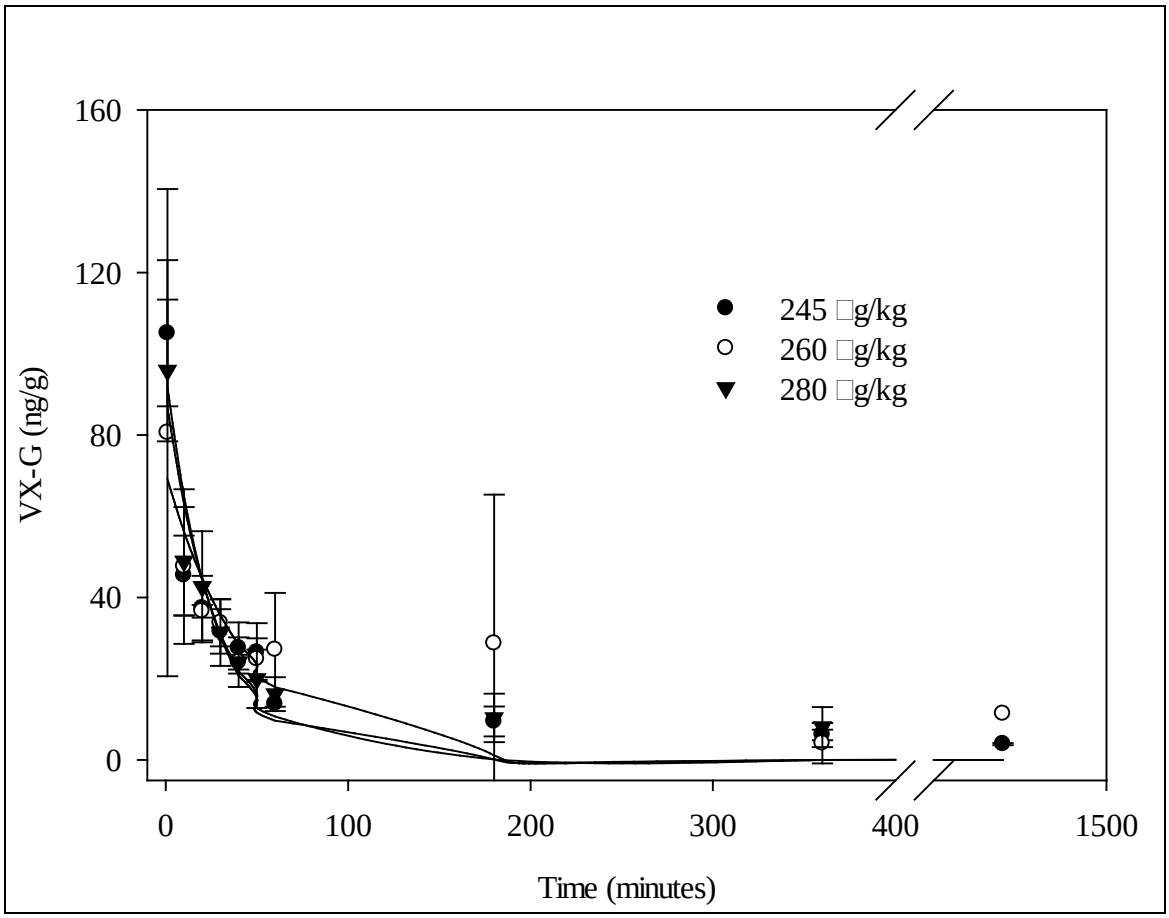

Figure 3. VX-G concentration in blood as a function of time
for three different doses of P(+)-VX.

Table 9. Pharmacokinetic Parameters from Serial Samplings Following Intravenous Exposure to Various Doses of P(+)-VX, P(–)-VX, and a Racemic Mixture of VX

Stereoisomer	**Dose (µg/kg)**	***k*** **(min^{-1})**	***t*½** **(min)**
P(+)-VX	245.0	0.0166	42
	260.0	0.0099	70
	280.0	0.0354	20
P(–)-VX	3.0	0.0011	635
	4.0	0.0010	694
	4.2	0.0009	802
	5.0	0.0012	578
Racemic VX mixture	3.7	0.0007	1,001
	4.1	0.0007	939
	4.8	0.0009	729
	5.0	0.0010	720
	6.0	0.0009	803
	7.0	0.0008	825

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ACRONYMS AND ABBREVIATIONS

BDL	below detection limit
CAS	Chemical Abstracts Service
CE	collision energy
CI	chemical ionization
ECBC	U.S. Army Edgewood Chemical Biological Center
GC	gas chromatography
IACUC	Institutional Animal Care and Use Committee
IPA	2-propanol
IS	internal standard
ISx	insufficient amount of sample
k	elimination rate constant
KF	potassium fluoride
LD_{50}	median lethal dose
MRM	multiple reaction monitoring
MS	mass spectrometry
MS/MS	tandem mass spectrometry
m/z	mass-to-charge ratio
NB	laboratory notebook
NMR	nuclear magnetic resonance
R^2	coefficient of determination
SPE	solid-phase extraction
$t_{½}$	half-life
VX	*O*-ethyl *S*-(2-diisopropylaminoethyl) methylphosphonothioate
VX-G	*O*-ethyl methylphosphonofluoridate, EA 1207

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